

WE CLAIM:

1. A method for detecting an endonuclease site polymorphism (ESP) in DNA, the method comprising:

- (a) isolating sample DNA;
- (b) deriving a set of concomitantly amplifiable target DNA fragments from the sample DNA;
- (c) treating the target DNA fragments obtained in step (b) with a probe restriction endonuclease reagent;
- (d) amplifying the probe restriction endonuclease reagent treated target DNA fragments of step(c);
- (e) analyzing the DNA of step (d) to determine which target fragments are amplified and/or which target fragments are not amplified; and wherein target DNA fragments which are amplified lack a recognition site for the probe restriction endonuclease reagent and target fragments having a recognition site for the probe restriction endonuclease reagent are not amplified.

2. The method of claim 1 the concomitantly amplifiable target DNA fragment of step (b) are derived by treatment of the sample DNA with a sampling restriction endonuclease reagent.

3. The method of claim 2 wherein the concomitantly amplifiable DNA fragments of step (b) are derived from sample DNA by treatment of the sample DNA with a first and a second restriction endonuclease reagent.

4. The method of claim 3 wherein said first restriction endonuclease reagent has a recognition sequence of six or more nucleotides and the second restriction endonuclease reagents has a recognition sequence of four or fewer nucleotides.

5. The method of claim 3 or 4 wherein said concomitantly amplifiable target DNA fragments are derived by step wise treatment of said sample DNA with the

first and the second restriction endonuclease reagents.

5 6. The method of claim 1 further comprising preparing of PCR primers which flank the endonuclease site polymorphism (ESP) for use in amplifying said concomitantly amplifiable target DNA fragments.

10 7. The method of claims 1, 2, 3, and 4 wherein the concomitantly amplifiable DNA fragments are modified by ligation of adapters to both termini of said fragments, and wherein said adapters are capable of serving as primers for amplification.

15 8. The method of claim 5 wherein the concomitantly amplifiable DNA fragments are modified by ligation of adapters to both termini of said fragments, and wherein said adapters are capable of serving as primers for amplification.

9. The method of claim 1 wherein the probe restriction endonuclease reagent of step (c) has a recognition sequence comprising six or more nucleotides.

20 10. The method of claim 1 wherein the probe restriction endonuclease reagent of step (c) has a recognition sequence comprising four or more nucleotides.

11. The method according to claim 1 wherein the probe restriction endonuclease of step (c) has a recognition sequence of two nucleotides.

25 12. The method according to claim 1 wherein the order of the steps (b) and (c) are reversed or carried out simultaneously.

30 13. The method according to claim 1 wherein said endonuclease site polymorphism is an alteration in a concomitantly amplifiable target fragment giving rise to a nucleotide sequence that is recognized and cut by the probe restriction endonuclease reagent .

- 48 -

14. The method of claim 1 wherein said site polymorphism is an alteration in the nucleotide sequence of a concomitantly amplifiable target fragment which eliminates a recognition sequence for said probe restriction endonuclease reagent.

5 15. The method of claims 1, 2, 3 and 4 wherein said concomitantly amplifiable DNA fragments are amplified by a polymerase chain reaction.

16. The method of claim 5 wherein said concomitantly amplifiable DNA fragments are amplified by a polymerase chain reaction.

10 17. The method of claim 1 wherein amplified target fragments are identified by their ability to hybridize to cognate probe DNA fragments.

18. A method for obtaining probe DNA fragments for use in detecting endonuclease site polymorphisms, the method comprising:

- 15 20 25
- (a) isolating sample DNA;
 - (b) deriving a set of concomitantly amplifiable target DNA fragments from the sample DNA;
 - (c) selecting from the target DNA fragments, probe DNA fragments having an endonuclease site polymorphism (ESPs) for the probe restriction endonuclease.

19. The method of claim 17 wherein said probe DNA fragments are derived by digestion of sample DNA with one or more sampling restriction endonuclease reagents.

20. The method of claim 18 wherein probe DNA fragments are derived by digestion of a pool of sample DNAs obtained from one or more individuals of a species.

30 21. The method of claim 18 wherein the probe DNA fragments are derived

by digestion of a pool of sample DNAs obtained from 10 or more individuals of a species.

22. The method of claim 18 wherein the probe DNA fragments derived by digestion of a pool of sample DNAs obtained from a pool of 50 or more individuals of species.

23. The method of any one of claims 19-21 wherein said species is selected from the group consisting of procaryotic species and eucaryotic species.

24. A method for obtaining probe DNA fragments for use in detecting endonuclease site polymorphisms (ESP) comprising preparing synthetic oligonucleotides based on the nucleotide sequence of amplifiable target DNA fragments containing endonuclease site polymorphism(s).

25. A method for producing a microarray of probe DNA the method comprising:

- (a) isolating sample DNA;
- (b) deriving a set of concomitantly amplifiable target DNA fragments from the sample DNA;
- (c) selecting probe DNA fragments having restriction endonuclease site polymorphisms (ESPs) from the sample restriction endonuclease treated target DNA fragments of step (b); and
- (d) arraying the probe DNA fragments obtained in step (c) on a solid substrate in a predefined region by attaching the fragments to the substrate.

26. The method of claim 24 wherein the DNA fragments of step (b) are obtained by treating sample DNA with one or more sample restriction endonuclease reagents.

27. The method of claim 24 wherein the said probe DNA fragments of step

- 50 -

(d) are synthetic oligonucleotides which correspond to the concomitantly amplifiable target DNA fragments derivable from said sample DNA and containing an endonuclease site polymorphism (ESP).

5 28. The method of claim 25, 26 or 27 wherein the solid support is selected from a group consisting of a planar solid support, a bead, a sphere and a polyhedron.

 29. The method of claim 25 wherein the microarray comprises at least 2,000 probe fragments.

10 30. The method of claim 26 wherein the microarray comprises at least 2,000 sythetic ologonucleotides.

 31. The method of claim 27 wherein the microarray comprises at least 2,000 probe fragments.

 32. The method of claim 28 wherein the microarray comprises at least 2,000 probe fragments.

20 33. The method of claim 25 wherein the microarray comprises at least 20,000 probe fragments.

 34. The method of claim 26 wherein the microarray comprises at least 20,000 sythetic ologonucleotides.

25 35. The method of claim 27 wherein the microarray comprises at least 20,000 probe fragments.

30 36. The method of claim 28 wherein the microarray comprises at least 20,000 probe fragments.